

iGEM TU/e 2015

Biomedical Engineering

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InterLab Study: PCR purification

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1 PCR purification

Estimated bench time: 30 minutes

Estimated total time: 30 minutes

Purpose: Purifying the product obtained from a PCR reaction.

It is essential to work with gloves at all times to protect the DNA from DNase activity.

1.1 Materials

- Autoclaved Eppendorf tubes
- Autoclaved H₂O (nuclease free water)
- PCR tubes with PCR product
- Pipettes and tips
- QIAquick PCR Purification Kit
- MiniSpin centrifuge

1.2 Setup & Protocol

- Mix 1 volume of the PCR sample with 5 volumes of buffer PB.
- Load the sample on a QIAquick spin column which is inserted in a collection tube (with a maximum of 800 µL per run).
- Centrifuge the sample for 1 minute at 13,400 rpm. Weight-balance the sample well.
- Discard the flow-through.
- Wash the sample with 750 µl of PE buffer and centrifuge for 1 minute at 13,400 rpm.
- Discard the flow-through.
- Dry spin the sample for 1 minute at 13,400 rpm.
- Transfer the spin column to a new autoclaved Eppendorf tube.
- Load 42 µl of autoclaved H₂O on the column (pipette drops in the middle of the membrane, do not touch the membrane). Incubate for 1 minute and centrifuge for 1 minute at 13,400 rpm.
- The resulting elution product will contain purified PCR product.

2 Nanodrop

Estimated bench time: -

Estimated total time: 5 minutes start-up, 2 minutes per sample

Purpose: Determine the concentration of DNA samples.

You are working with DNA, so it is essential to work with gloves at all times to protect your plasmids from DNase activity.

2.1 **Materials**

- Autoclaved H₂O
- DNA samples
- NanoDrop spectrophotometer
- Pipettes and tips

2.2 **Setup & Protocol**

- Start the NanoDrop spectrophotometer.
- Select the DNA measurement 'Nucleic Acid' in the NanoDrop menu.
- Clean the surface of the NanoDrop with dH₂O.
- Perform a calibration and blank measurement by entering one drop of 2 µl autoclaved H₂O.
- Clean the surface again and place 2 µl per sample on the NanoDrop and measure the concentration. Write down the concentration (possibly on cryo-babies to stick on your DNA samples).